Role of maize stover incorporation on nitrogen oxide emissions in a non-irrigated Mediterranean barley field

Diego Abalos · Alberto Sanz-Cobena · Lourdes Garcia-Torres · Jan Willem van Groenigen · Antonio Vallejo

Abstract
Aims Agricultural soils in semiarid Mediterranean areas are characterized by low organic matter contents and low fertility levels. Application of crop residues and/or manures as amendments is a cost-effective and sustainable alternative to overcome this problem. However, these management practices may induce important changes in the nitrogen oxide emissions from these agroecosystems, with additional impacts on carbon dioxide emissions. In this context, a field experiment was carried out with a barley (Hordeum vulgare L.) crop under Mediterranean conditions to evaluate the effect of combining maize (Zea mays L.) residues and N fertilizer inputs (organic and/or mineral) on these emissions.

Methods Crop yield and N uptake, soil mineral N concentrations, dissolved organic carbon (DOC), denitrification capacity, N$_2$O, NO and CO$_2$ fluxes were measured during the growing season.

Results The incorporation of maize stover increased N$_2$O emissions during the experimental period by c. 105 %. Conversely, NO emissions were significantly reduced in the plots amended with crop residues. The partial substitution of urea by pig slurry reduced net N$_2$O emissions by 46 and 39 %, with and without the incorporation of crop residues respectively. Net emissions of NO were reduced 38 and 17 % for the same treatments. Molar DOC:NO$_3^-$ ratio was found to be a robust predictor of N$_2$O and NO fluxes.

Conclusions The main effect of the interaction between crop residue and N fertilizer application occurred in the medium term (4–6 month after application), enhancing N$_2$O emissions and decreasing NO emissions as consequence of residue incorporation. The substitution of urea by pig slurry can be considered a good management strategy since N$_2$O and NO emissions were reduced by the use of the organic residue.

Keywords Nitrous oxide · Crop residues · Incorporation · Greenhouse gas · Nitric oxide · Pig slurry

Introduction
Agricultural soils are one of the main anthropogenic sources of nitrous oxide (N$_2$O) and nitric oxide (NO) (IPCC 2007). Nitrous oxide accounts for c. 8 % of the current anthropogenic greenhouse effect (IPCC 2007), and is considered to be the dominant ozone-depleting substance emitted in the 21st century (Ravishankara et al. 2009). Nitric oxide is highly reactive in the atmosphere, contributing to its oxidizing capacity and more
specifically to the chemical formation of tropospheric ozone, thereby affecting human health and plant photosynthesis (Staffelbach et al. 1997). Agriculture is also associated with the greenhouse gas (GHG) carbon dioxide (CO₂), accounting for 1% of the global CO₂ emissions (OECD 2000). However, agriculture has a significant climate change mitigation potential which could change its position from one of the largest emitters to a much smaller emitter or even a net sink of GHGs (Bellarby et al. 2008). In this sense, because organic and mineral nitrogen (N) fertilizers are known to be key variables in the regulation of trace gas emissions from agricultural soils (IPCC 2007), the active management of agricultural practices associated with fertilization provides an opportunity for climate change mitigation (Smith and Conen 2004).

Returning crop straw to the soil has been proposed as a measure to increase soil fertility, as crop residues are a source of soil organic matter, which is the primary source of plant nutrients and an energy source for soil microorganisms (Malhi et al. 2011). Residue quality is the primary factor controlling decomposition rates and N release. Among the strongest determinant quality parameters are the C:N ratio and N, lignin and soluble polyphenol contents (Palm and Rowland 1997). Crop residues with C:N ratios greater than 20, such as those of cereals, are difficult to break down and usually decompose more quickly when N fertilizer is applied (Snyder et al. 2009). Therefore, adding a complementary source of N (mineral or organic) when crop residues are incorporated into the soil could stimulate straw mineralization increasing N-use efficiency and producing higher yields (Garcia-Ruiz and Baggs 2007).

A mechanism by which the interactive effect of crop residues and N fertilizers can affect N gaseous emissions was suggested by Vanlauwe et al. (2001). The application of N-fertilizer can create high levels of available N that exceed plant demand, leading to potential N losses as gaseous emissions (e.g. N₂O and NO). Incorporation of straw into the soil may reduce these emissions by inducing N-fertilizer immobilization. This temporary immobilization delays N release until later in the season, and thereby may serve to match the rate of soil N supply with the rate of plant N uptake. As evidence for this mechanism, in an incubation experiment, Gentile et al. (2008) found a reduction of fertilizer-derived N₂O emissions when mineral fertilizer was combined with low quality (C:N ratio >42:1; lignin content <15%; polyphenol content <4%) maize (Zea mays L.) residue.

Conversely, some studies have shown that crop residue incorporation can increase N₂O emissions from soil (e.g. Huang et al. 2004; Frimpong and Baggs 2010). The release of organic C after incorporation of cereal residues having a high C:N ratio may provide the energy for denitrification, resulting in an increase of N₂O emissions, particularly where N availability is high after N-fertilizer application (Sarkodie-Addo et al. 2003). Little is known about the combined effect of N fertilizer and crop residue addition on NO emissions from croplands, due to a lack of field studies involving both factors (Yao et al. 2009; Liu et al. 2011).

Alternative N sources for use in agricultural production need to be examined to allow replacement or substitution of synthetic N-fertilizer use (i.e. urea), a product of fossil fuel based energy. Nitrogen sources which are available in large quantities in many countries are animal manures, including pig slurries, from intensive livestock production facilities (Meade et al. 2011). It has been shown that the types of C (easily mineralizable and resistant organic C) and N (NO₃⁻, NH₄⁺ and organic N) added to the system through animal manures may interact with the N cycle, affecting the N₂O/NO production/consumption processes (Chadwick et al. 2000).

The objective of this study was to evaluate the effect of applying maize residues and fertilizer inputs (organic and/or mineral), combined or alone, on nitrogen oxide emissions under field conditions during a barley (Hordeum vulgare L.) crop. An additional objective was to relate these emissions with crop N uptake; analyzing N₂O and NO emissions on a yield basis provides information for estimating the environmental impacts of intensive agricultural production systems (van Groenigen et al. 2010). We hypothesized that the release of C from the crop residues would increase N₂O emissions but that this increase may be offset by a correspondingly higher yield. To gain a better understanding of the effect of the management practices tested on the overall GHG budget, CO₂ emissions were also analyzed during the experimental period.

Materials and methods

Soil characteristics

The field experiment was carried out at “El Encin” Field Station (40°32’N, 3°17’W) in Alcalá de Henares (Madrid), located in the Henares river basin. According
to the USDA soil taxonomy system (Soil Survey Staff 1992), the soil is a Calcic Haploxerert with a clay loam texture in the upper horizon (0–28 cm). Some physicochemical properties of the top soil layer were: total organic C, 8.2±0.4 g kg⁻¹; pH_H2O 7.6; bulk density, 1.4±0.1 g cm⁻³; CaCO₃, 13.1±0.3 g kg⁻¹; clay, 28%; silt, 17%; sand, 55%; total N, 0.75±0.12 g kg⁻¹. The 10-year mean temperature was 13.2 °C and the mean annual rainfall over the same period, 430 mm.

Experimental design

Eighteen plots (8×8 m) were selected and arranged in a randomized complete block design with two factors, crop residue management practices (remove (-R) or retain (+R)), and fertilizer type (control without N-fertilizer application (C), pig slurry + urea (PS+U), and urea (U)). There were three replicates of every treatment.

The harvested maize straw from the previous crop was cut into pieces of 10–20 cm, homogenized, quantified and spread or removed, depending on the treatment, on 10th November 2010. Sub samples were collected on the same day for laboratory analysis. The maize straw was applied at a rate equal to 5,149 kg C ha⁻¹, corresponding to the average straw yield from the previous maize crop. On 15th November 2010, all plots received a blanket basal application of 50 kg P ha⁻¹ as triple superphosphate and 50 kg K ha⁻¹ as potassium sulfate by broadcasting and incorporating the fertilizers into the upper 10 cm of the soil using a roto-vator.

Pig slurry was obtained from a treatment plant at Almazán (Soria, Spain). A rotary sieve drum (0.9 mm mesh) was used to separate the solid and liquid fractions of the manure. Separation of the solid fraction reduced the viscosity and so facilitated distribution and infiltration into the soil. The characteristics of the applied liquid pig slurry were: total N, 3.1 g kg⁻¹; total C, 18.02 g kg⁻¹; DOC, 0.5 g kg⁻¹; NH₄⁺-N, 2.3 g kg⁻¹; pH, 7.51 and dry matter, 16 g kg⁻¹.

The application of fertilizers was adjusted to provide 150 kg total N ha⁻¹ during the crop period, in two separate applications. Before sowing (16th November 2010), 50 kg N ha⁻¹ were applied as urea or pig slurry depending on the treatment. The remaining 100 kg N ha⁻¹ were applied as urea for all fertilized treatments, as a top-dressing (25th February 2011). Pig slurry was applied to the soil using a waterering can, connected by a hosepipe to a 100 L tank, to produce a uniform distribution on the soil surface. An exact weight of urea was broadcast by hand. Immediately after the first fertilizer application, a roto-vator was used to incorporate the maize straw, slurry and urea into the upper soil layer (0–10 cm), and the experiment started. Barley (Hordeum vulgare L. cv Bornova) was sown on 25th November 2010 by direct drilling, at a rate of 350 seeds m⁻². The field was kept free of weeds, pests and diseases, following local practices (i.e. herbicides). Harvesting took place on 20th June 2011.

To facilitate analysis, our data acquisition was divided into two different periods according to the fertilizer applications. The first fertilization covered the period from the incorporation of the maize straw in mid-November, to the crop GS 25, in mid-February. The period from mid-February to harvest, hereafter is referred to as the second fertilization.

Sampling and analysis of gases

Nitrous oxide and CO₂ fluxes were measured using the closed chamber technique (Clayton et al. 1994) with opaque manual chambers of 19.34 L (diameter 35.6 cm, height 19.3 cm). The chambers were closed by fitting them into stainless steel rings which were inserted into the soil to a depth of 10 cm to minimize lateral diffusion of gases. Measurements were always made with the barley plants inside the chambers. When the plants were higher than 19 cm, plastic intersections of 19 cm covered with Teflon were used between the ring and the chamber. Gas samples (vol. 20 mL) were taken by syringe 0, 30 and 60 min after chamber closure and collected in evacuated gas chromatography vials. Thermometers were placed inside three randomly selected chambers during the closure period of each measurement in order to correct the fluxes for temperature.

Concentrations of N₂O and CO₂ were quantified by gas chromatography, using a HP-6890 gas chromatograph (GC) equipped with a headspace auto-analyzer (HT3), both from Agilent Technologies (Barcelona, Spain). HP Plot-Q capillary columns transported gas samples to a ⁶³Ni electron-capture detector (ECD) to analyze N₂O concentrations. CO₂ was reduced by a methanizer and then analyzed by a flame ionization detector (FID). Gas samples were taken from the chambers four times during
the first and second weeks after fertilizer applications, then two to three times per week during the following month, and subsequently on a weekly basis until the end of the crop period.

To measure NO fluxes, a gas flow-through system was used. The chamber used for this analysis had the same characteristics as that used for GHGs sampling, but it was covered with Teflon and provided with inlet and outlet holes and a transparent lid (Sanchez-Martin et al. 2010a). During this measurement, air (filtered through a charcoal and aluminium/KMnO₄ column to remove O₃ and NO₃) was passed through the headspace of the chamber and gas samples were pumped from the chambers at a constant flow rate of 40 L min⁻¹ to the detection instruments through Teflon tubing. NO was analyzed by a chemiluminescence detector (AC31M-LCD, Environnement S.A., Poissy, France). An ambient air sample was taken and measured between each gas sampling. For the gas sampling, concentrations were measured continuously until they gave a constant value; this normally took approximately 5 min per sample. The NO flux was calculated as described in Sanz-Cobena et al. (2012). Intensive NO measurements were carried out during the first month following fertilizer applications in order to study the highest emissions associated with the different treatments. After the first month, NO emissions were measured monthly.

Denitrification capacity

Laboratory incubations were undertaken to investigate the impact of carbon availability on N₂O emissions. For this purpose we measured the denitrification capacity of soils collected on two sampling dates. The first soil sample was taken 16 days after the residue incorporation (26 November), and the second sample was taken when the highest N₂O emission peak of the measurement period was observed (30 March), 1 month after the second fertilizer addition. Three replicates for each treatment were used. Denitrification capacity was measured according to the technique described by Yeomans et al. (1992) with a few modifications. Into 250 mL glass bottles, 30 g of fresh soil and 12.5 mL of a solution containing 42.9 mM KNO₃ were added. The bottles were sealed with a rubber stopper, repeatedly flushed with N₂ (10 min) and separated into two groups (with or without a further addition of acetylene 8 %). After 0, 6 and 12 h, gas samples (vol. 10 mL) were taken and stored in evacuated vials. Nitrous oxide was analyzed by gas chromatography as previously described.

Soil and crop residue analysis

Every day of gas sampling, soil samples were taken from the upper layer (0–10 cm) of the soil, in order to relate gas emissions to soil properties. Four soil cores were randomly sampled close to the ring for each plot and mixed in the laboratory to make the sample homogeneous. Soil NO₃⁻-N and dissolved organic carbon (DOC) were determined by extracting 8 g of fresh soil with 60 mL of deionized water. An Orion 720A NO₃-electrode (Thermo Fisher Scientific, Beverly, MA, USA) was used to analyze NO₃⁻-N and DOC content was determined as described by Mulvaney et al. (1997). NH₄⁺-N concentrations were analyzed by extracting 8 g of fresh soil with 50 mL of KCl (1 M) and measured by automated colorimeter (AAII Auto-analyzer, Technicon Hispania, Madrid, Spain). Water-filled pore space (WFPS) was estimated by dividing the volumetric water content by total soil porosity. Total soil porosity was calculated by measuring the bulk density of the soil according to the relationship: soil porosity = 1 – (soil bulk density/2.65); assuming a particle density of 2.65 Mg m⁻³ (Danielson and Sutherland 1986). Volumetric water content was determined by oven-drying soil samples at 105 °C. Rainfall and temperature data were obtained hourly from a meteorological station located at the field site. A temperature probe inserted 10 cm into the soil was used to measure soil temperature.

At maturity, the yield of grain and aboveground biomass were measured by randomly harvesting four 0.5×0.5 m squares from each plot. The plant components were dried in a 65 °C oven, weighed and ground for determination of N concentration (AOAC 2000).

The physicochemical properties of the crop residue are shown in Table 1. According to these properties, the maize residues were classified as Class III, and so are advised to be applied in combination with N fertilizer (Gentile et al. 2008). Total N was assessed as described previously. Cellulose, hemicellulose and lignin were determined from neutral detergent fibre (Mertens 2002), acid detergent fibre and acid detergent lignin (AOAC 2000). Total soluble polyphenols
Table 1 Crop residue (maize stover) characteristics

<table>
<thead>
<tr>
<th>Total N (g kg⁻¹)</th>
<th>Total C (g kg⁻¹)</th>
<th>C/N</th>
<th>Lignin (%)</th>
<th>Cellulose (%)</th>
<th>Hemicellulose (%)</th>
<th>Polyphenols (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.81±0.12</td>
<td>487.32±0.13</td>
<td>127.81±0.15</td>
<td>4.47±0.46</td>
<td>32.14±2.39</td>
<td>30.45±4.29</td>
<td>1.45±0.06</td>
</tr>
</tbody>
</table>

Means ± standard deviation of 12 replicates.

were determined by the Folin-Ciocalteau method (Constantinides and Fownes 1994).

Calculations and statistical analysis

Cumulative \(N_2O\)-N, NO-N and CO₂-C emissions per plot were calculated by linear interpolations between sampling dates. It was assumed that emissions followed a linear trend during the periods in which no samples were taken. The emission factors (EF) of applied nitrogen fertilizer for \(N_2O\) and NO were calculated using the total emissions from the fertilized treatments and the unfertilized field plots during the entire measurement period. Yield scaled \(N_2O\) and NO emissions were calculated based on van Groenigen et al. (2010), considering total N uptake (i.e. by grain and straw) and cumulative \(N_2O\) and NO emissions, respectively.

Statistical analysis was performed using Statgraphics Centurion XVI. The data distribution normality of the fluxes (\(N_2O\), NO and CO₂) and of soil \(NH_4^+\), \(NO_3^-\) and DOC was verified using the Kolmogorov-Smirnov test. In some cases, the data were log-transformed before analysis. Differences between treatments at each sampling event and in the cumulative emissions in normally distributed data were analyzed using analysis of variance (two-way ANOVA, \(P<0.05\)), being the factors crop residue incorporation and nitrogen fertilization (i.e. Residue and Fertilizer). The Least Significant Difference (LSD) test was used for multiple comparisons between means. For non-normally distributed data, the Kruskal-Wallis test was used on non-transformed data to evaluate differences at \(P<0.05\). When significant effects were found to occur, Schai–Hamerle analysis was carried out as post hoc test. Linear regression analyses were performed to determine the relationships between \(N_2O\)-N, NO-N and CO₂-C fluxes and soil \(NH_4^+\)-N, \(NO_3^-\)-N and DOC and between WFPS and soil temperature. Non-linear regressions were used to determine the best fit equation between \(N_2O\) and NO emissions and the molar DOC: \(NO_3^-\) ratio.

Results

Environmental conditions

During the first fertilization period, soil moisture ranged from 59 % to 75 % WFPS, with a high mean value of 68 %, mainly due to the continuous precipitations over the winter season (Fig. 1). Average daily soil temperature was 5.8 °C. The incorporation of crop residues did not show any effect on soil temperature.

Average daily soil temperature was c. 7.5 °C after the second fertilization in February, with minimum air temperatures below 0 °C and highest of 22 °C in May. WFPS decreased to 41 % after 16 days in this period and then ranged between 34 % and 77 % until harvest. No significant differences between treatments (\(P>0.05\)) were found in soil WFPS at any sampling time.

Changes in soil mineral N and DOC

Organic and mineral fertilizers significantly increased (\(P<0.05\)) the ammonium and nitrate concentrations in the topsoil (0–10 cm) with respect to the control plots, for at least 30 days after application (Fig. 2a–d). No statistically significant differences (\(P>0.05\)) were seen for \(NH_4^+\) and \(NO_3^-\) concentrations from fertilized plots, either with and without residue incorporation, for the first fertilization period. Soil \(NH_4^+\) content varied between 0.5 and 40.7 mg \(NH_4^+\)-N kg⁻¹ during this initial period. The largest soil \(NO_3^-\) value was measured in the U (–R) plots (22 mg \(NO_3^-\)-N kg⁻¹). Similarly, no statistically significant differences (\(P>0.05\)) were found between fertilized treatments for soil \(NH_4^+\) and \(NO_3^-\) concentrations during the second fertilization period. In this period, soil \(NH_4^+\) and \(NO_3^-\) contents ranged between 0.4 and 94.7 mg \(NH_4^+\)-N kg⁻¹ and between 0.3 and 37.8 mg \(NO_3^-\)-N kg⁻¹, respectively.

Dissolved organic carbon ranged from 5.2 to 56.8 mg C kg⁻¹ during the crop period. The application of pig slurry significantly increased (\(P<0.05\)) the DOC concentration in comparison to U and C plots.
during the first fertilization period, both with and without residue incorporation (Fig. 2c, f). Incorporation of crop residues in November significantly increased ($P<0.05$) the DOC concentrations of fertilized plots (i.e. U and PS+U) at the moment of the second fertilizer application.

Fluxes of nitrous oxide

Nitrous oxide emissions were significantly increased ($P<0.05$) by adding fertilizers and crop residues, with several emission peaks occurring during the experimental period (Fig. 3a, b). The highest emission peaks of the winter season were observed for all fertilized treatments 24 days after N fertilization. The U treatments showed the largest fluxes (1.7 and 1.5 mg $N_2O$-N m$^{-2}$ day$^{-1}$), with and without crop residues added, respectively. After these peaks, $N_2O$ fluxes decreased, ranging from 0 to 0.5 mg $N_2O$-N m$^{-2}$ day$^{-1}$ for all treatments, with no significant differences between them ($P>0.05$).

In the second fertilization period, daily $N_2O$ fluxes significantly ($P<0.01$) increased over a period of 44 days (i.e. from 17th March to 30th April) from fertilized plots in comparison to C. Throughout this period, $N_2O$ emissions from plots with residue incorporation were significantly higher ($P<0.05$) than those from plots without residue incorporation, with the highest peaks measured for U (+R) (3.8 mg $N_2O$-N m$^{-2}$ day$^{-1}$) and PS+U (+R) (3.2 mg $N_2O$-N m$^{-2}$ day$^{-1}$). Afterward, $N_2O$ fluxes decreased sharply for all treatments. The soil acted as a sink (i.e. negative fluxes) for $N_2O$ at some sampling events, especially for C (−R). Positive
correlations were found between N\textsubscript{2}O emission and three soil parameters, NO\textsubscript{3}^{-}-N ($r=0.21$, $P<0.05$, $n=108$), NH\textsubscript{4}^{+}-N ($r=0.20$, $P<0.05$, $n=108$) and soil temperature ($r=0.20$, $P<0.05$, $n=108$). During the second fertilization period, a statistically significant positive correlation was found between N\textsubscript{2}O and DOC ($r=0.20$, $P<0.05$, $n=108$).

When each period was considered independently, cumulative N\textsubscript{2}O emissions were higher in the second fertilization period (c. 80 % of total annual emission). Total cumulative emissions during the experimental period were greater from U (+R) (1.29 kg N\textsubscript{2}O-N ha\textsuperscript{-1}) and PS+U (+R) (0.76 kg N\textsubscript{2}O-N ha\textsuperscript{-1}) than from the other
treated plots (Table 2), although this increase in emissions was found to not be statistically significant ($P>0.05$).

Fluxes of nitric oxide

A significant increase ($P<0.05$) in the NO emission rate was observed in all fertilized plots within 19 days following the first fertilizer application (Fig. 3c, d). In this period, the largest fluxes were measured 6 and 8 days after fertilization, from the U (−R) (8.3 mg NO-N m$^{-2}$ day$^{-1}$) and the U (+R) (8.6 mg NO-N m$^{-2}$ day$^{-1}$) treated plots, respectively. After 20–25 days NO fluxes remained below 0.5 mg NO-N m$^{-2}$ day$^{-1}$ in all treatments, and occasionally negatives fluxes were measured. During the second fertilization period, the largest fluxes of NO were associated with the PS+U (−R) treated soils (15.6 mg NO-N m$^{-2}$ day$^{-1}$) indicate standard errors. The continuous arrows indicate the date of fertilizer application and the dotted arrow indicates the incorporation of crop residues

21 days after fertilization. Nitric oxide fluxes were significantly correlated with N$_2$O ($r=0.23$, $P<0.05$, $n=84$), NO$_3^-$-N ($r=0.42$, $P<0.001$, $n=84$) and NH$_4^+$-N ($r=0.25$, $P<0.05$, $n=84$).

Cumulative NO-N emissions were significantly higher from urea fertilized plots without crop residue incorporation (1.39 kg NO-N ha$^{-1}$), than that of the other treatments (Table 2).

Fluxes of carbon dioxide

Respiration rates were lower than 1,000 mg CO$_2$-C m$^{-2}$ day$^{-1}$ during the first fertilization period (Fig. 4). The highest peak was measured on 19th January for the PS+U (+R) treatment (937 mg CO$_2$-C m$^{-2}$ day$^{-1}$), and the lowest on 16th December, for U (+R) (9 mg CO$_2$-C m$^{-2}$ day$^{-1}$) and U (−R) (10 mg CO$_2$-C m$^{-2}$ day$^{-1}$). Emissions increased sharply after the second fertilization for all treatments,
Table 2 Cumulative N₂O-N, NO-N and CO₂-C emissions over the different periods of the experiment

<table>
<thead>
<tr>
<th>Crop residue</th>
<th>Fertilizer</th>
<th>First fertilization</th>
<th>Second fertilization</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N₂O (g N₂O-N ha⁻¹)</td>
<td>NO (g NO-N ha⁻¹)</td>
<td>CO₂ (kg CO₂-C ha⁻¹)</td>
<td>N₂O (g N₂O-N ha⁻¹)</td>
</tr>
<tr>
<td>+ R</td>
<td>8.4a</td>
<td>140.0a</td>
<td>150.2a</td>
<td>142.7ab</td>
</tr>
<tr>
<td>PS+U</td>
<td>90.3a</td>
<td>286.6ab</td>
<td>325.3b</td>
<td>678.5bc</td>
</tr>
<tr>
<td>U</td>
<td>249.5b</td>
<td>478.5c</td>
<td>354.5be</td>
<td>1046.8c</td>
</tr>
<tr>
<td>− R</td>
<td>−6.3a</td>
<td>147.0a</td>
<td>276.8ab</td>
<td>3.3a</td>
</tr>
<tr>
<td>C</td>
<td>56.0a</td>
<td>322.5b</td>
<td>241.2ab</td>
<td>356.3ab</td>
</tr>
<tr>
<td>PS+U</td>
<td>232.6b</td>
<td>477.9c</td>
<td>326.1b</td>
<td>440.3abc</td>
</tr>
</tbody>
</table>

Different letters within columns indicate significant differences by applying the Least Significant Difference (LSD) test at P<0.05. *, **, ***: Significant at the 0.05, 0.01 and 0.005 probability level, respectively.

being significantly higher from the plots with crop residue incorporation (P<0.05). The largest flux was observed for U (+R) (7.384 mg CO₂-C m⁻² day⁻¹) on 28th March. The CO₂ emissions correlated significantly with soil temperature (r=0.47, P<0.001, n=108), N₂O (r=0.49, P<0.005, n=108) and NO emissions (r=0.23, P<0.05, n=84).

Total cumulative emissions from N fertilized plots were significantly higher (P<0.005) than that from the C plots, being the highest emissions measured for U (+R) and PS+U (+R), of 3,208 and 3,102 kg CO₂-C ha⁻¹ respectively (Table 2).

Denitrification capacity

Following fertilizer application and residue incorporation in November, denitrification capacity was significantly higher (P<0.05) in the PS+U (+R) plots compared to all other treatments. After the second fertilization, mean denitrification capacity was significantly lower (P<0.05) in the N fertilized plots without residue incorporation, than that of the plots with residue incorporation (Fig. 5). Urea fertilized soils with residue incorporation (i.e. U (+R)) had the highest denitrification capacity on 30th March.

Relationship between N₂O and NO emissions with molar DOC:NO₃⁻ ratio

Most of the significant N₂O pulses occurred when the molar DOC:NO₃⁻ ratio was close to the unity. Negative N₂O fluxes were measured when the ratio was above 2. The best-fit equation for these data (Fig. 6a) was y=0.27 x^{−0.432} (r=0.51, P<0.001, n=90). Nitric oxide emission pulses (> 2 mg NO-N m⁻² day⁻¹) were observed when the ratio was lower than 2 (Fig. 6b). Statistical analyses showed that y=(3.388)−0.542 x was the empirical model for these variables (r=0.53, P<0.005, n=52).

Discussion

Agricultural soils in semiarid Mediterranean areas are characterized by low organic matter contents, and low fertility levels mainly due to a warm and dry climate and the cultivation system employed (Garcia-Gil et al. 2000). Application of crop residues and/or manures as amendments is a cost-effective and sustainable alternative to improve the organic matter content of these soils. However, as demonstrated by this study, these management practices may induce important changes in some of the processes leading to trace gas emissions from these agroecosystems.

The incorporation of maize straw increased the N₂O emissions during the experimental period by c. 105 %. This effect was significant during the second period of our study (P<0.05), when the emissions from the U and PS+U plots amended with crop residues were 138 and 90 % higher, respectively, than that for the same fertilizer treatments without residue incorporation (Table ).
Fig. 4 Fluxes of CO₂ during the experimental period. Data are provided separately for treatments a without and b with crop residue incorporation. Vertical lines indicate standard errors.

These significantly higher emissions were most likely due to a higher denitrification capacity stimulated by the C substrate added with the maize straw. The availability of both organic C and NO₃⁻ regulates denitrification (Taylor and Townsend 2010). In our laboratory study under anaerobic and non-limiting NO₃⁻ conditions, the denitrification capacity was significantly higher for the plots amended with crop residues 140 days after its addition (Fig. 5). This may reflect a higher C availability in these plots as the progressive increase of DOC content after 30 days in the plots where the residues were incorporated would seem to suggest (Fig. 2f). Another factor promoting the higher N₂O emissions from these plots was found from an analysis of the CO₂ fluxes. The higher CO₂ emissions of the crop residue amended plots may be indicative of greater respiration (Table 2). This may have created more anaerobic microsites in the soil (Drury et al. 1991), thus increasing N₂O production through denitrification.

The addition of maize straw did not immobilize the soil mineral N. Crop residues with C:N ratio less than 30 are expected to result in net N mineralization, while those with C:N ratios higher than 30, as is generally the case in cereal straw, cause immobilization (Alexander 1977). Cayuela et al. (2009) found that wheat straw (C:N = 198) and cotton cardings (C:N = 30.5) led to a rapid immobilization of N that affected microbial biomass size and activity, and subsequent N mineralization. Similarly, sorghum stover (C:N = 72) and maize straw (C:N = 32) resulted in N immobilization during incubation experiments (Das et al. 1993; Hadas et al. 2004). Immobilization of soil N may decrease N₂O emissions due to decreased availability of ammonium and nitrate for the processes of nitrification and denitrification (Baggs et al.)

Fig. 5 Mean values for denitrification capacity. Significant differences (P<0.05) between treatments are indicated by different upper-case (26 November) or lower-case (30 March) letters.
This immobilization of soil N can be partially reduced if N fertilizer is added (Yao et al. 2009). This is probably because at high N fertilizer rates there is adequate N for both crop and microorganisms, as observed by Malhi et al. (2001). In our study, this effect is indicated by similar sizes of the soil mineral N pool between fertilized plots with and without residue incorporation (Fig.2a, b, c, d), similarly as found by others (e.g. Liu et al. 2011).

Nitric oxide emissions were reduced in the plots amended with crop residues. The reduction was particularly significant $(P<0.01)$ during the second period of the experiment, likely as a consequence of increased NO consumption through denitrification processes (Skiba and Ball 2002). The ratio $\text{NO}/\text{N}_2\text{O}$ is an indicator for distinguishing the prevalence of nitrification or denitrification (e.g. Sanz-Cobena et al. 2012). Anderson and Levine (1986) found that the molar ratio of $\text{NO}/\text{N}_2\text{O}$ was usually greater than unity for nitrifiers and much less than unity for denitrifiers. During the emission pulse of the second fertilization period, c. 74 and 51 % of $\text{NO}/\text{N}_2\text{O}$ ratios from fertilized treatments, with and without crop residues respectively, were $<1$. Therefore, in the maize stover amended plots, these gases could have been mainly produced through the denitrification process. Under these conditions, NO reduction (i.e. consumption by denitrifiers) was enhanced, leading to reductions in the emissions of this gas. This may show an indirect soil C influence over NO emissions. Further evidence of this effect is shown in Fig. 6b. When the molar $\text{DOC:NO}_3^-$ ratio was above 2, the emission pulses of NO were significantly reduced. This could be due to the use of NO as electron-acceptor by denitrifying organisms under $\text{NO}_3^-$ limiting conditions.

The addition of crop residues did not have any significant effect on $\text{N}_2\text{O}$ and NO emissions during the first fertilization period (Table 2). One reason for this may be, as Heal et al. (1997) explained, that substrates with C:N ratios higher than 75, as is the case in maize straw, are difficult to break down by soil microbiota. Additionally, sub-optimal soil temperatures in winter could also contribute to a lower decomposition rate. Considering that the $\text{N}_2\text{O}$ and NO emission pulse occurred in a period of c. 20 days after the first fertilization, there was probably insufficient time for biological decomposition of the crop residues within this initial period. This may suggest that laboratory studies, which generally last less than 50 days (e.g. Aulakh et al. 1991; Flessa and Beece 1995; Huang et al. 2004), may lead to biased conclusions regarding the effect of crop residues on gaseous emissions from soils.

The cumulative $\text{N}_2\text{O}$ emissions of the control treatment (i.e. without either residue or fertilizer addition) were $-3.01 \text{ g N}_2\text{O-N ha}^{-1}$. Therefore, these plots acted as a sink for $\text{N}_2\text{O}$ during the experimental period. Net $\text{N}_2\text{O}$ consumption has previously been measured in agricultural systems (Yamulki et al. 1995; Merino et al. 2004). The main $\text{N}_2\text{O}$ consumption pathway is likely to be denitrification, in which $\text{N}_2\text{O}$ may be reduced to $\text{N}_2$. As discussed previously, denitrifying bacteria tend to utilize oxidized forms of N such as $\text{NO}_3^-$ as electron-acceptor before utilizing $\text{N}_2\text{O}$ or NO.

![Fig. 6](image-url) Regression of $\text{a N}_2\text{O and b NO}$ on $\text{DOC:NO}_3^-$ ratio. The inset panels show the low values more clearly. The intercept of the dash line on the X-axis represents the molar ratio of $\text{DOC:NO}_3^-$ equated to 2.
(Yamulki and Jarvis 2002). In the control plots, the availability of mineral N was limited throughout the experimental period but not the C substrate (Fig. 2). These conditions promote N₂O consumption (Sanchez-Martin et al. 2010b). Most of the negative N₂O fluxes occurred when the molar DOC:NO₃⁻ ratio was above 2 (Fig. 6a). These results suggest that from this value of 2, denitrifiers use N₂O and NO for their catabolic processes. The incorporation of crop residues to the control plots without N fertilization depleted this sink effect, with cumulative emissions from this treatment (i.e. C (+R)) of 150.9 g N₂O-N ha⁻¹.

Some studies have shown that a mitigation strategy to reduce N₂O and NO emissions is the substitution of urea by pig slurry at pre-plant fertilization (Vallejo et al. 2006; Meijide et al. 2007). Pig slurry application at sitedress is infrequent among farmers due to the heavy equipment needed for its incorporation which can damage the root system of growing crops. In this study, the partial substitution of urea by pig slurry (i.e. PS+U compared to U) reduced net N₂O emissions by 46 % from the plots where the crop residues were incorporated, and by 39 % from the plots without residue incorporation. In the case of net NO emissions, 38 and 17 % reductions were measured for the same treatments, respectively. As shown by others, the most likely mechanisms through which pig slurry reduced N₂O and NO emissions were by significantly reducing the N₂O/NO ratio (Dittert et al. 2005) and promoting the NO reduction (Sanchez-Martin et al. 2008), respectively. The lower emissions could also be due to a lower mineral N level in the pig slurry treatments.

The N₂O emission factors, ranging from 0.28 % to 0.76 % of the applied N, are below the IPCC default value (i.e. 1 %), suggesting that national inventories may overestimate actual N₂O emissions from these type of agroecosystems. In the case of NO, the average emission factor (0.64 %) was comparable to the worldwide average (0.7 %) for fertilized croplands (Bouwman et al. 2002). This study provides the first dataset on yield-scaled N₂O and NO emissions in relation to the management practices tested under Mediterranean conditions. Our yield-scaled N₂O emissions are within the range reported as optimal by van Groenigen et al. (2010) (5–16 g N₂O-N kg⁻¹ above-ground N uptake). However, the N surpluses (i.e. applied N minus above-ground N uptake) were always higher than those recommended by these authors (0–50 kg N ha⁻¹), suggesting that agroecosystems-specific relationships are needed to better link agronomic productivity to environmental sustainability. Taking into account the possible long-term soil carbon sequestration by adding residues, which has both agricultural and climate change mitigation aspects, caution must be exercised establishing general recommendations for farmers based on the incorporation of maize residues. In our study, such addition significantly increased N₂O (c. 112 %) and slightly decreased NO (c. 28 %) yield-scaled emissions (Table 3). Although higher yields (8.5 %) and N uptakes (24 %) were obtained from the fertilized plots with residue incorporation, contrary to our hypothesis, these increases were not large enough to offset the corresponding increase in N₂O emissions.

The influence of crop residues on trace gas emissions have been traditionally studied using a wide range of residues. However, farmers usually do not have amendments available other than the residues obtained from a previous crop. Taking into account that a typical crop rotation includes maize followed by

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Yield (Mg ha⁻¹)</th>
<th>N uptake (kg N ha⁻¹)</th>
<th>EF (%)</th>
<th>Yield-scaled emissions (g N₂O-N or NO-N kg⁻¹ N uptake)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Straw</td>
<td>Grain</td>
<td>Total</td>
<td>Straw</td>
</tr>
<tr>
<td>C (+R)</td>
<td>0.64a</td>
<td>0.80a</td>
<td>1.44a</td>
<td>4.2a</td>
</tr>
<tr>
<td>PS+U (+R)</td>
<td>2.83b</td>
<td>3.24b</td>
<td>6.08b</td>
<td>24.4bc</td>
</tr>
<tr>
<td>U (+R)</td>
<td>2.94b</td>
<td>3.46b</td>
<td>6.40b</td>
<td>31.1c</td>
</tr>
<tr>
<td>C (-R)</td>
<td>0.79a</td>
<td>0.98a</td>
<td>1.77a</td>
<td>5.0a</td>
</tr>
<tr>
<td>PS+U (-R)</td>
<td>2.62b</td>
<td>3.06b</td>
<td>5.68b</td>
<td>15.9b</td>
</tr>
<tr>
<td>U (-R)</td>
<td>2.71b</td>
<td>3.10b</td>
<td>5.82b</td>
<td>21.3bc</td>
</tr>
</tbody>
</table>

Different letters within columns indicate significant differences by applying the Least Significant Difference (LSD) test at $P<0.05$
barley or wheat, the assessment made in our study can be considered as representative of a common and affordable management practice. Particularly when considering that the use of crop residues to conserve soil and water in arid and semiarid areas is becoming more and more important (Lampurlanés and Cantero-Martínez 2006).

The evaluation of pig slurry as an important N source with environmental implications is of major interest in countries with high swine populations. In this study, the partial substitution of urea by pig slurry significantly reduced net N$_2$O and NO emissions either when the crop residues were incorporated or not. An interesting finding was that when the emissions were linked to yield data, this positive effect of pig slurry was reduced for N$_2$O and almost disappeared for NO (Table 3). However, the substitution of urea by pig slurry can be considered a good management strategy since N$_2$O and NO yield-scaled emissions were not enhanced by the use of the organic residue. As a consequence, we recommend routine reporting of crop N uptake rates in N loss studies to translate general findings into fertilizer recommendations.

Conclusions

This study highlights the key role of C added with maize stover residues in the emissions of N$_2$O and NO from soils with a low organic C content. The incorporation of crop residues increased the N$_2$O emissions (c. 105 %) due to the stimulation of the denitrifying activity by providing soil microorganisms with available carbon in the presence of nitrate. However, NO emissions were significantly reduced in the plots amended with crop residues, likely as a consequence of increased NO consumption through denitrification. Both N$_2$O and NO fluxes were highly dependent on the soil DOC:NO$_3^-$ ratio. An affordable mitigation strategy to reduce these emissions was found to be the partial substitution of urea by pig slurry. The addition of pig slurry decreased net N$_2$O and NO emissions by 43 and 27 %, respectively.

Acknowledgements The authors are grateful to the Spanish Ministry of Science and Innovation and the Autonomous Community of Madrid for their economic support through Projects AGL2009-08412-AGR and the Agrisost Project (S2009/AGR-1630), respectively. Jan Willem van Groenigen is supported by a personal VIDI grant from the Netherlands Organization of Scientific Research/Earth and Life Sciences (NWO-ALW). This study would not have been possible without technical assistance from the technicians and researchers at the Department of Chemistry and Agricultural Analysis of the Agronomy Faculty (Technical University of Madrid, UPM). Special thanks to Simon Jeffery for his critical comments on a previous version of the manuscript.

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